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Reduction of Saltiness and Bitterness After a Chlorhexidine Rinse

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Abstract

Chronic rinsing with chlorhexidine, an oral-antiseptic, has been shown to decrease the saltiness of NaCl and the bitterness of quinine. The effect of acute chlorhexidine on taste has not been investigated. The purpose of the present study was to examine the effect of acute chlorhexidine rinses on taste intensity and quality of 11 stimuli representing sweet, salt, sour, bitter and savory. All stimuli were first matched for overall intensity so the effects of chlorhexidine would be directly comparable across compounds. As a control treatment, the bitter taste of chlorhexidine digluconate (0.12%) was matched in intensity to quinine HCl, which was found to cross-adapt the bitterness of chlorhexidine. Subjects participated in four experimental conditions: a pre-test, a quinine treatment, a chlorhexidine treatment, and a post-test condition, while rating total taste intensity and taste qualities in separate test sessions. Relative to the quinine treatment, chlorhexidine was found to decrease the salty taste of NaCl, KCl and NH_4Cl, and not to significantly affect the tastes of sucrose, monosodium glutamate (MSG), citric acid, HCl and the taste of water. The bitter taste of urea, sucrose octa-acetate and quinine were suppressed after chlorhexidine rinses relative to water rinses, but were only marginally suppressed relative to quinine rinses. Potential mechanisms are discussed.

Introduction

Psychophysical studies using pharmacological blockers of specific taste qualities (e.g. bitterness or sweetness) hold the potential to provide insight into both the type and number of transduction mechanisms. For this technique to highlight a particular component of taste physiology, two prerequisites must be met. First, the agent must specifically block a taste quality or qualities and not taste in general. Second, the pharmacological agent must have a known biochemical action that could be effective on taste physiology.

The best example of specific taste blockade is sodium-specific taste reduction in some rodents that are exposed orally to the compound amiloride HCl (Halpern, 1998). Amiloride was initially targeted as a candidate for blocking salt taste because: (i) it blocks selected epithelial sodium channels in the kidney and elsewhere (Sonnenberg *et al.*, 1987; Kopp *et al.*, 1998) and (ii) it was hypothesized that these channels mediated salt taste (Schiffman *et al.*, 1983; Heck *et al.*, 1984). Subsequent non-human studies have supported this hypothesis in some species/strains of rodents (Bernstein and Hennessy, 1987; Hill *et al.*, 1990; McCutcheon, 1991; Spector *et al.*, 1996; Harada *et al.*, 1997; Miyamoto *et al.*, 1998; Roitman and Bernstein, 1999), but not others (Ninomiya *et al.*, 1989; Tonosaki and Funakoshi, 1989; Gannon and Contreras, 1995; Miyamoto *et al.*, 1998 1999). For a review see Halpern (Halpern, 1998).

In one of the more elegant uses of taste blockers, rats not only failed to distinguish among NaCl and KCl solutions after oral amiloride treatment, but also came to respond to NaCl solutions, in taste guided instrumental responses, as if they tasted like KCl (Spector *et al.*, 1996). From this we can infer that amiloride interferes with an NaCl transduction mechanism that is critical to the recognition of NaCl (e.g. the amiloride-sensitive sodium channel), but does not greatly impede the recognition of KCl. Furthermore, in these animals, NaCl appears also to stimulate the same or a similar mechanism as does KCl, which is largely unaffected by amiloride. The salty taste of sodium salts in humans, however, is not blocked by amiloride, as appears to be the case with several strains/species of rodent, e.g. mice (Halpern, 1998).

In humans, initial reports suggested that topical amiloride altered the taste of NaCl, partially decreasing its intensity on the tip of the tongue (Schiffman *et al.*, 1983; McCutcheon, 1992; Tennissen, 1992; Smith and Ossebaard, 1995; Tennissen and McCutcheon, 1996; Anand and Zuniga, 1997). Subsequent studies showed that while amiloride does reduce the overall perceived intensity of NaCl, amiloride does not reduce the perceived saltiness. Rather, amiloride was reported to decrease the subtle sour side-quality of NaCl (Ossebaard and Smith, 1996, 1997; Ossebaard *et al.*, 1997; Halpern and Darlington, 1998). Thus, despite the ability of amiloride to block the unique characteristics of sodium taste in some rodents, it does not have this effect in humans. We believe, however, that Schiffman's (Schiffman *et al.*, 1983) hypothesis that salty taste transduction in mammals occurs via the direct passage

of cations into taste cells through cation channels is true of humans as well, although the human salty taste channel remains to be characterized.

If amiloride does not selectively block salty taste in humans, are there any other pharmacological substances that might block saltiness? There are reports that chronic use of the topical oral disinfectant and anti-gingival agent, chlorhexidine (at 0.12–0.2%), specifically reduces the salty taste of NaCl (Lang *et al.*, 1988) or both the salty taste of NaCl and the bitter taste of quinine HCl (Helms *et al.*, 1995). Whether these specific effects might also be observed with an acute chlorhexidine oral rinse is the focus of the present paper.

Since previous reports showed that chlorhexidine decreased the saltiness of NaCl and the bitterness of quinine, we included three salts (of varying cation) and three bitter-tasting compounds, as well as a sweetener (sucrose), an umami/savory stimulus (glutamic acid, sodium salt—MSG), both organic and inorganic sour stimuli (citric and hydrochloric acid) and water in the stimulus array. All stimuli were matched for total intensity in order to make valid comparisons across compounds, since in most cases taste blockers are more effective on weaker taste stimuli and less effective on stronger stimuli. In addition, since 0.12% chlorhexidine has a very strong bitter taste, a bitter-tasting control compound that cross-adapts the bitterness of chlorhexidine was necessary.

Experiment 1

The purpose of this experiment was to identify a bitter tasting compound that (i) could be matched in intensity to 0.12% chlorhexidine and (ii) cross-adapts the bitterness of chlorhexidine. Pilot testing suggested that quinine HCl would cross-adapt chlorhexidine digluconate, so the experiment focused on this bitter-tasting compound as a control. We wished to obtain a control-treatment stimulus that would cross-adapt chlorhexidine because chlorhexidine, in previous reports, may have reduced bitterness or saltiness because of cross-adaptation effects rather than its pharmacological properties. Therefore, a bitter control compound was desired that possessed similar cross-adaptation properties to chlorhexidine.

Materials and methods

Subjects

Thirteen non-smoking volunteers (mean age 27 ± 3.4 years) participated in the study. All participants (five women, eight men) were involved in a preliminary session to determine the ability to taste all stimuli to be presented. Subjects were generally representative of the University City area of Philadelphia (primarily Caucasian and African American). Subjects volunteered and provided signed consent on an Institutional Review Board (IRB) approved form. All subjects were compensated for their participation.

Stimuli

Chlorhexidine digluconate (CHX) (Sigma) and quinine HCl (Fluka) were the stimuli. QHCl was intensity matched to the 0.12% CHX. CHX was chosen at the 0.12% level because this concentration is commonly used to kill oral bacteria in the treatment of gingivitis and its prophylaxis and because this concentration has been reported to alter taste perception following chronic use (Schaupp and Wohnaut, 1978; Helms *et al.*, 1995).

Stimulus delivery

An aliquot of 10 ml of each solution was presented in 30 ml polyethylene medicine cups (Baxter). Subjects were instructed how to sample in a practice session. Subjects were asked to sample ~10 ml of each stimulus, rate it and expectorate. Sampling duration was restricted to 5 s per presentation in order to minimize adaptation effects.

Training

Subjects were prescreened for their ability to assign a bitterness intensity rating to each. Bitter taste intensity was measured with the 'Labeled Magnitude Scale' (LMS) (Green *et al.*, 1993, 1996). This scale is partitioned by verbal descriptors of intensity that we commonly use in everyday language, including: no sensation, barely detectable, weak, moderate, strong, very strong, and strongest imaginable. The subjects rated the intensity of the taste stimuli by indicating on the scale the position closest to the appropriate descriptor using a computer and mouse. Subjects were told first to determine which descriptor most appropriately described the intensity of the sensation, then fine tune their rating by moving the cursor to the proper location between that descriptor and the next one. Subjects were also told to rate the stimuli relative to other taste sensations of all kinds that they have experienced in daily life. We repeatedly emphasized that the top of the scale is the 'strongest imaginable' oral sensation, which includes intense oral pain. A single LMS intensity rating was used for intensity scale testing.

Intensity matching

Intensity matching was done over several days and all subjects participated. Each matching session involved administering a concentration of QHCl or 0.12% CHX. Because CHX was determined to rapidly self-adapt, ratings were made on the LMS for only one stimulus per day. After repeated testing and adjustments of the QHCl concentrations, intensity means were calculated of the QHCl concentration that seemed closest to the CHX ratings for all subjects on their last three trials. The bitterness of the mean QHCl rating was compared to the mean value of the standard CHX. The mean QHCl intensity was deemed acceptable for further testing if it was approximately within 5% of standard CHX intensity. QHCl at 0.01 M was selected as the best matched concentration for the subject pool. This concentration of QHCl is approximately two and

half orders of magnitude above detection threshold for most subjects.

Test procedure

The following test procedure was used to validate the pilot study by determining whether 0.12% CHX was similar in bitter intensity to 0.01 M QHCl and whether QHCl would cross-adapt the bitterness of CHX after several exposures to QHCl. There were four testing sessions conducted in a counterbalanced ABAB format. Subjects were either asked (A) to rate the bitterness of a 0.12% CHX 5 s rinse on the LMS in one session to obtain baseline bitterness for CHX, or, to test for QHCl adaptation, they were asked (B) to hold 10 ml of 0.01 M QHCl in their mouth over four consecutive 30 s exposures followed by a 5 s CHX rinse. This QHCl adaptation procedure (B) involved first rinsing with de-ionized water twice. Then a 10 ml solution of QHCl was held in the mouth for 30 s and expectorated. At 30 s, a second 10 ml solution of the same compound was then immediately taken and held for another 30 s. This was done two more times for a total of four 10 ml QHCl rinses over two consecutive minutes. After the first 5 s of each QHCl rinse and the 5 s CHX rinse, the bitterness was rated on the LMS.

Data analysis

The data were analysed for skewness of distribution and were found to be log-normal, as is customarily found with LMS data (Green *et al.*, 1996). Therefore, all data were log-transformed for analyses and presented graphically as geometric means (GeoMeans) ± geometric standard errors (GeoSEs). GeoSEs were calculated as the difference between the geometric mean and the antilog of the arithmetic mean + SE and arithmetic mean – SE of the base 10 logged data, i.e. [$10^{(\text{mean of the logged data + SE of the logged data})}$ _ GeoMean]; [GeoMean – $10^{(\text{mean of the logged data – SE of the logged data})}$]—see Bishop *et al.* (Bishop *et al.*, 1975) for methods of estimating variance in logged distributions. The bitterness intensity data were analysed with a two-way repeated measures ANOVA with six levels for the condition factor (two CHX ratings and four QHCl ratings) and two levels for the repetition factor. In addition, QHCl self-adaptation was analysed separately for the four QHCl ratings. If there was a significant main effect, then post-hoc pair-wise comparisons with Scheffé's test were conducted. Specific attention was paid to the comparison between the CHX baseline and the first quinine rating (intensity matching), the four quinine ratings (self-adaptation), and the CHX ratings before and after quinine adaptation (cross-adaptation). The criterion for significance in all post-hoc pair-wise comparisons was set to a *P*-value of 0.05.

Results

The bitterness of the chlorhexidine and the quinine were not significantly different (compare bars 1 and 2, Figure 1) ($P = 0.38$). The geometric mean bitterness was rated between near strong (between strong and very strong for the arithmetic mean). QHCl bitterness ratings decreased with each subsequent rinse, declining overall by ~25%; self-adaptation was evident between the first and fourth quinine ratings (compare bars 2 and 5, Figure 1) [$F(3,36) = 2.85$, $P < 0.05$]. QHCl also significantly cross-adapted the bitterness of CHX (compare bars 1 and 6, Figure 1) [$F(5,60) = 12.20$, $P < 0.00001$). There was no main effect of repetition ($P = 0.46$). Note that the terminal CHX rating occurred after the fourth QHCl rinse, while the terminal QHCl rating occurred closer to the beginning of the fourth QHCl rinse, which may, in part, account for differences between the terminal QHCl and CHX ratings (bars 5 and 6).

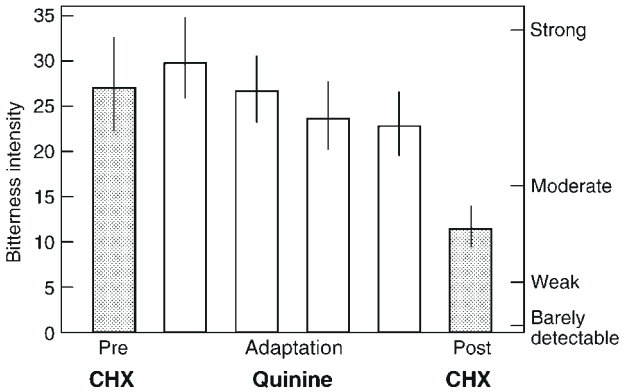

Figure 1 This figure depicts the results of the cross-adaptation procedure in Experiment 1. The bitterness of 0.12% chlorhexidine digluconate (CHX) (gray bars) and 10mM quinine HCl (QHCl) (open bars) was plotted as a function of number of exposures to QHCl. Data are presented as geometric means ± geometric standard errors (GeoSEs). Notice that upward-deflecting GeoSEs are greater than the downward-deflecting GeoSE (see Experiment 1 Analysis for explanation). The first gray bar and the first white bar (measured on separate sessions) were preceded only by water. Each subsequent white bar was preceded by one, two and three 30 s QHCl presentations, respectively, and the second gray bar was preceded by four 30 s QHCl presentations. The ticks on the right vertical axis display the position of the verbal markers on the Labeled Magnitude Scale.

Discussion

The two objectives of Experiment 1 were met. QHCl was successfully intensity matched to the bitterness of CHX on average and it cross-adapted to CHX. This allowed us to control for the bitterness of CHX as a factor in modifying other tastes (particularly other bitter-tasting compounds) and to employ a compound that presumably shared a common physiological basis assuming that compounds that cross-adapt share factors (Froloff *et al.*, 1998).

Experiment 2

CHX at 0.12% was employed as a pre-rinse to determine if it would modify the taste of 11 test stimuli. QHCl at 0.01 M was used as a control bitter pre-rinse based upon the results of Experiment 1. Stimuli were also rated for baseline taste levels before any pre-rinses (PRE) and after both pre-rinses

(POST) in separate sessions at the beginning and end of the experiment. The taste intensities of the test stimuli were matched for intensity to 0.3 M NaCl. Each subject was tested for his or her unique set of intensity matches. Therefore, a different set of the 11 stimuli was employed for each subject (see Table 1). In all four conditions (PRE, QUININE, CHX, POST), ratings of total intensity and of the intensity of individual qualities, including salt, sweet, bitter, sour and savory, were collected in separate test sessions.

Materials and methods

Subjects

A total of 16 nonsmoking volunteers (mean age 26 ± 4.3) participated in the study. All participants (11 women, five men) were involved in a preliminary session to determine the ability to taste all stimuli to be presented. This included delivery of each stimulus and asking the participant to accurately describe the qualities perceived. Subjects volunteered and provided signed consent on an IRB approved form. All subjects were compensated for their participation.

Stimuli

A total of 11 stimuli were selected to represent a range of taste qualities. They were: NaCl (Fisher), KCl (Sigma), NH_4Cl (Aldrich), urea (Sigma), sucrose octa-acetate (SOA, Sigma), QHCl (Fluka), sucrose (Sigma), MSG (Sigma), citric acid (Sigma), HCl (Sigma) and deionized, Millipore™ filtered water. In an attempt to ensure that the stimuli (except water) were of approximately equal strength, intensity matching was performed using a 0.3 M NaCl standard. Inosine 5′-monophosphate (IMP, Sigma) (50 mM) was used during the training phase in mixture with MSG for reasons describedbelow. Chlorhexidine (Sigma) was chosen again at the 0.12% level for reasons given in Experiment 1.

Stimulus delivery

Aliquots of 20 ml of each solution were presented in 30 ml polyethylene medicine cups (Baxter). Subjects were instructed how to sample in a practice session at the beginning of training. Subjects were asked to sample ~10 ml of each stimulus and rate it, then proceed to check their response by sampling the remaining solution. Sampling duration was restricted to 5 s per 10 ml sampling in order to minimize adaptation effects.

Training

Subjects were trained how to identify each of the five qualities by presenting exemplars to them. Salty taste was identified as the predominant quality from 10 ml of 150 mM NaCl, bitterness was identified as the predominant quality from 0.05 mM QHCl, sweetness as the predominant quality from 300 mM sucrose, sourness as the predominant quality from 3 mM citric acid, and savory was the dominant taste quality from a mixture of 100 mM MSG and 50 mM inosine 5′-monophosphate (IMP). This mixture was employed to demonstrate savory taste because MSG alone is both savory and salty, whereas the mixture is considerably more savory than salty. In all cases, the exemplar was said to have the specified quality as the dominant quality, but may also elicit other qualities to a lesser degree. Subjects were instructed to focus attention on the dominant taste quality.

Subjects were prescreened for their abilities (i) to assign an intensity rating to each stimulus and (ii) to rate the intensity of each quality using separate quality scales. In order to fulfill (i) and (ii), two different software programs were utilized both in the screening and the test sessions. Taste intensity was measured with a single LMS intensity rating, as described for Experiment 1.

The second computerized data-collection program was used in the quality scale test sessions of the experiment. This program presented five LMS scales on a single screen for each stimulus, rather than one single LMS scale for overall intensity. Each one of the five LMS scales was labelled one of the following: SWEET, SALTY, SOUR, SAVORY or BITTER. Subjects were asked to rate the intensity of the qualities of each stimulus by moving the cursor to the appropriate level on each scale. Subjects were also told that some stimuli could be rated on multiple scales if, for example, both sourness and bitterness were perceived from a single solution. The process of selecting the appropriate level for each taste quality on the LMS was the same as in the intensity scale testing. The order of the five scales on the monitor was randomized from session to session, but remained constant within each test session.

Intensity matching

Intensity matching was carried out over several weeks and used 300 mM NaCl as the standard. Each matching session involved administering a low concentration of the previously mentioned nine stimuli (water was not included) and the NaCl. Subjects rated each of these stimuli, including the standard NaCl solution, using the LMS. There was a 2 min interval between each stimulus delivery during which subjects rinsed with water at least twice. After repeated testing and adjustments of each individual's stimulus concentrations, means were calculated of the three to six terminal ratings given for respective solution concentrations. The number of terminal tests deemed necessary for the 'final' concentration adjustment was based upon the variability of the intensity ratings for the particular concentration being tested. The solution intensity means were then compared to the mean value of the standard NaCl solution (0.3 M). Each individual's matched solution mean intensities were deemed acceptable for further testing if they were within 5% of the sodium standard's intensity. See Table 1 for individual intensity matches to the 11 stimuli.

Test procedure

There were five sections of the experiment: intensity matching/training (of all subjects); pre-test; quinine test; CHX test; and post-test. The pre-test was used to gather

Table 1 The individual intensity matches of all ten compounds in each of the 16 subjects are listed in rows as molar solutions

ID	NaCl	KCl	NH_4Cl	Urea	SOA	Quinine	Sucrose	MSG	Citric acid	HCl
1	0.3	0.269	0.0852	2.301	8.008E–5	2.017E–6	0.795	0.729	0.0082	0.0073
2	0.3	0.215	0.0426	1.918	8.008E–5	1.344E–6	0.53	0.648	0.0061	0.0024
3	0.3	0.215	0.0852	2.685	8.008E–5	3.362E–6	1.855	1.296	0.0020	0.0012
4	0.3	0.220	0.126	1.918	1.802E–4	3.352E–5	1.55	0.901	0.0038	0.0054
5	0.3	0.269	0.1491	1.151	1.401E–5	1.344E–6	1.06	0.81	0.0051	0.0018
6	0.3	0.323	0.1704	0.767	8.008E–5	1.344E–6	1.325	1.134	0.0041	0.0043
7	0.3	0.215	0.0426	0.384	2.002E–5	2.017E–6	0.53	0.162	0.0042	0.0024
8	0.3	0.323	0.0852	0.384	2.002E–5	1.344E–6	1.06	0.162	0.0020	0.0049
9	0.3	0.43	0.128	3.068	2.002E–4	1.681E–5	1.06	0.486	0.0082	0.0122
10	0.3	0.323	0.171	2.301	4.004E–5	1.344E–6	0.53	0.486	0.0082	0.0098
11	0.3	0.43	0.128	3.068	1.201E–4	2.017E–6	1.59	0.486	0.0042	0.0049
12	0.3	0.215	0.171	3.835	2.002E–4	1.681E–5	1.06	0.81	0.0061	0.0024
13	0.3	0.215	0.128	0.767	8.008E–5	2.017E–6	0.53	0.486	0.0020	0.0012
14	0.3	0.323	0.171	3.068	1.201E–4	1.344E–6	0.53	0.486	0.0082	0.0024
15	0.3	0.323	0.0426	1.534	1.602E–4	3.362E–5	0.80	0.648	0.0041	0.0024
16	0.3	0.215	0.128	3.068	1.602E–4	1.681E–5	1.59	0.81	0.0082	0.0049
Mean	0.3	0.283	0.1159	2.014	1.022E–4	8.567E–6	1.025	0.659	0.0053	0.0044
SE	0	0.019	0.0117	0.272	1.585E–5	2.862E–6	0.112	0.077	0.0006	0.0008

The mean values and standard errors are given below each column for the specified compound.

baseline perceptions of the 11 chemicals presented with no pre-rinse. The quinine test involved administering quinine as a pre-rinse prior to delivery of the compounds. The CHX test used CHX as the pre-rinse. The post-test mirrored the pre-test and its purpose was to determine whether there were any long-term changes in response to stimuli due to repeated CHX and/or quinine rinses from the previous test sessions. Subjects alternated pre-rinsing with chlorhexidine and quinine on different days and the order was counterbalanced across subjects on any given day (half with one compound, half with the other). Thus, half the pre-rinse sessions began with CHX and half with quinine, and CHX exposures occurred at a minimum of every 48 h. Previous reports suggest that after repeated chlorhexidine use including three applications per day for 8 days, taste sensitivity fully recovered 48 h after the last CHX rinse (Schaupp and Wohnaut, 1978).

In all there were eight computerized sessions that were completed using one of two types of LMS ratings (not counting the training session or the intensity matching sessions), four measuring total intensity and four measuring quality intensities. Included within each of the two pre-tests, CHX tests, quinine tests and post-tests, were an intensity scale session and a quality scale session (one type of scaling per day). Each stimulus was rated twice within each test session, presented in random order without replacement (e.g. 2, 1, 5, 4, 3; 1, 3, 5, 2, 4). Subjects were asked to abstain from eating for 2 h prior to each session.

In the pre-test and post-test sessions, a subject would take ~10 of a 20 ml sample into their mouth, initially rate it while holding it, expectorate, and then rinse twice. The other 10 ml was then immediately sampled to allow subjects to double-check the responses that had just been given. Subjects would repeat this procedure until they had sampled every solution. Subjects were asked to rinse at least twice with deionized water in between every rating. The computerized timed-break between ratings was 1 min 45 s. This ensured that all stimuli would be presented in a test session within 50 min of the end of the oral pre-rinsing regimen. This interval was selected because a pilot study revealed that chlorhexidine's pre-treatment effects began to wear off after ~50 min. Other researchers have also observed this recovery period (Schaup and Wohnaut, 1978; Bota *et al.*, 1984).

The CHX test and quinine test differed from the pre-test and post-test in only one-way. An initial pre-rinse was administered before the procedure described above was completed. The pre-rinse involved either a 0.12% CHX solution (CHX test) or a 0.01 M QHCl solution (quinine test). Pre-rinsing involved first rinsing with deionized water twice. Then a 10 ml solution of CHX or QHCl was held in the mouth for 30 s and expectorated. At 30 s, a second 10 ml solution of the same compound was then immediately taken and held for another 30 s. This was done twice more for a total of four 10 ml solutions over two consecutive minutes. After the 2 min pre-rinse followed 1 min of no activity, during which no water rinsing was allowed. Following the rest period was a 4 min period in which the subject was required to rinse at least four times in order to remove most residual pre-rinse solution from the oral cavity. After the 4 min of water rinsing were completed, subjects continued testing as in the pre-test and post-test sessions.

Data analysis

The total intensity data and the quality intensity data were analysed separately. For each data set, analysis of variance (ANOVA) was carried out on the logged data. The data were analysed for skewness of distribution and were found to be log-normal. All data were therefore computed as geometric means. The total intensity data were analysed with a two-way repeated measures ANOVA by compound. One factor was the test condition with four levels (PRE, QUININE, CHX, POST) and the second factor was the repetition of ratings, with two levels. Compounds were analysed individually so as not to lose statistical power by making spurious comparisons. If there was a significant main effect, then post-hoc pair-wise comparisons with Scheffé's test were conducted. Specific attention was paid to the comparison between the PRE and QUININE conditions, the PRE and the CHX conditions, and the PRE and POST conditions. This last comparison determines whether responses were stable over time. If both QHCl and CHX were found to alter a given solution, then the inter-treatment (QUININE–CHX) comparison was reported to demonstrate whether one effect was greater than another. To control for multiple ANOVA comparisons for the 11 stimuli, the criterion for significance in all post-hoc pair-wise comparisons was set to a P-value of $P = 0.05/11$ or $P = 0.00455$.

For the quality data the same ANOVA strategy was applied. In this case, however, the risk of losing statistical power was even greater than with total intensity because of the multiple qualities that were measured. Therefore, two-way ANOVAs were performed only on the primary qualities for each stimulus which were: NaCl—salty; KCl—salty, bitter; NH_4Cl—salty, bitter, sour; urea—bitter, sour; SOA—bitter; QHCl—bitter, sour; citric acid—sour, bitter; HCl—sour, bitter; MSG—savory, salty. To control for multiple ANOVA comparisons for the ten stimuli and 17 quality tests, the criterion for significance in all post-hoc pair-wise comparisons was set to a P-value of $P = 0.05/17$ or $P = 0.00294$.

In order to increase statistical power, we also conducted three-way repeated measures ANOVAs on the group of three bitter compounds and also on the three salts. These tests were made for both total intensity data and for bitter taste quality of the bitter compounds and salty taste quality of the salts. One factor was pre-rinse treatment (pre, QHCl, CHX), a second was salt or bitter stimulus identity (NaCl, KCl, NH_4Cl/urea, SOA, QHCl), and the third was repetition (two repetitions).

No subjects were dismissed in this experiment.

Results

Total intensity

There was a main effect of treatment on the intensity of NaCl [$F(3, 45) = 10.37$, $P < 0.0001$] and no effect of repetition (Figure 2). Pair-wise comparisons revealed that CHX significantly reduced the intensity of NaCl ($P < 0.0001$) while QHCl did not. There were no main effects of treatment on the intensity of KCl or NH_4Cl. Because CHX pre-rinses appeared to decrease slightly the saltiness of all three salts (Figure 2), a separate ANOVA was conducted on just the salts to increase power of the test. QHCl pre-rinses did not significantly decrease the intensity of any salt relative to the pre-test condition. CHX significantly suppressed the intensity of NaCl relative to both the pre-test condition and the QHCl pre-rinse condition ($P < 0.001$). A post-hoc test for pre-rinse conditions (pooled across stimuli and repetition) showed that CHX pre-rinses tended to reduce the intensity of all the salts at a borderline significance level ($P < 0.05$) and QHCl did not ($P = 0.944$). The CHX pre-rinse suppression of saltiness was greater than that of QHCl pre-rinses ($P < 0.01$).

There was a main effect of treatment on the intensity of SOA [$F(3,45) = 11.87$, $P< 0.00001$] and QHCl [$F(3,45) = 8.04$, $P < 0.001$] and no effects of repetition (Figure 2). Pair-wise comparisons revealed that CHX pre-rinses significantly reduced the intensity of SOA and QHCl ($P < 0.0001$) while QHCl pre-rinses did not. There was a trend for the QHCl pre-rinse to reduce the SOA ($P = 0.21$) and the QHCl ($P = 0.08$) intensity relative to the PRE-ratings but this was not significant. Because of this trend, a separate ANOVA was conducted on just the bitter compounds. While QHCl pre-rinses did not significantly decrease the intensity of any one bitter compound relative to the pre-test condition, the difference between the QHCl pre-rinse effect and the CHX pre-rinse effect on the QHCl stimulus was not significantly different ($P = 0.93$). This means that QHCl pre-rinses self-adapted the QHCl stimulus to an intermediate degree. A post-hoc test for pre-rinse conditions (pooled across stimuli and repetition) showed that QHCl pre-rinses tended to reduce overall intensity of the three bitter compounds to a borderline significance level ($P = 0.051$) and CHX suppressed their intensity significantly ($P < 0.00001$). The overall CHX pre-rinse suppression of bitter compound intensity was greater than that of QHCl pre-rinses ($P < 0.01$).

There were no significant effects of either pre-rinse on the total intensity of KCl, NH4Cl, urea, sucrose, MSG, citric acid, HCl or deionized, filtered water. The intensity of KCl, NH_4Cl and urea were shown to be slightly decreased when pooled within compound category, e.g. salts or bitter compounds.

Intensity of qualities

Salts. There was a main effect of treatment on the saltiness of NaCl [$F(3,45) = 15.47$, $P < 0.00001$] and KCl [$F(3,45) = 18.76$, $P < 0.00001$] (Figure 3) and no effects of repetition. Pair-wise comparisons revealed that CHX significantly reduced the saltiness of NaCl and KCl ($P < 0.00001$) while QHCl did not. There were no effects of either treatment on the bitterness of KCl. There was a strong trend for CHX

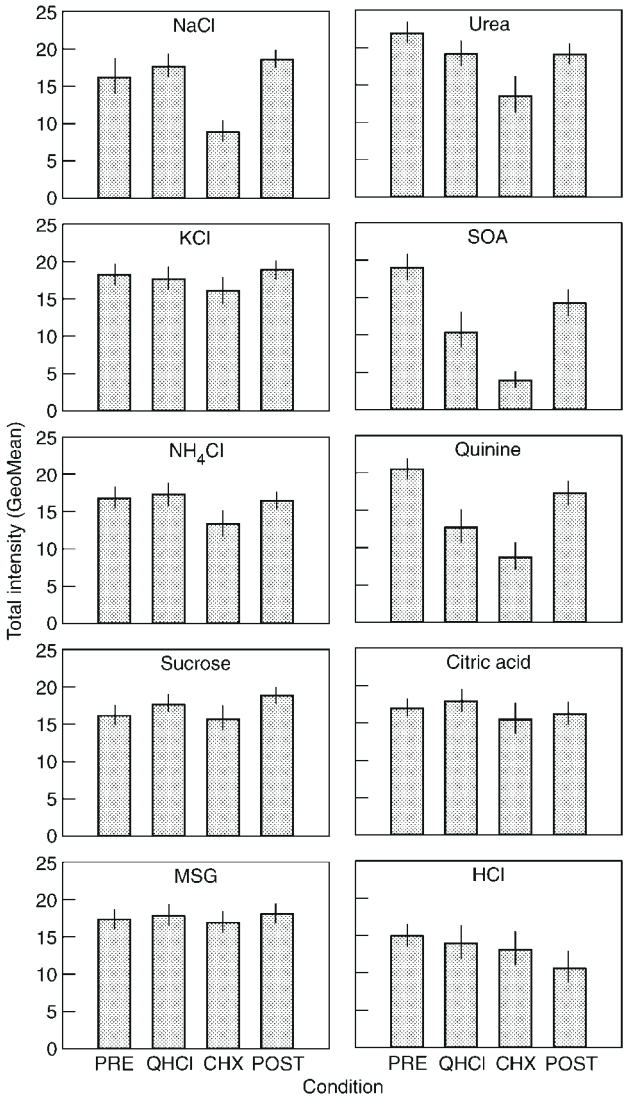

Figure 2 The total taste intensities of all ten compounds are depicted in ten separate panels. In each panel the vertical axis represents the geometric mean total intensity ± geometric SEs and the horizontal axis depicts the four testing conditions. The PRE condition was with no pre-rinse, before any pre-rinsing had occurred; QHCl was with four 30 s 10mM QHCl pre-rinses; CHX was with four 30 s 0.12% CHX pre-rinses; POST was after both counter-balanced pre-rinses had occurred. The PRE condition established baseline responses and the POST condition established chronic effects of QHCl and CHX rinses. Each condition occurred on separate test sessions, see text for methods. The first column of panels consists of the salts and the second column consists of the bitter tasting compounds for the first three rows of panels only.

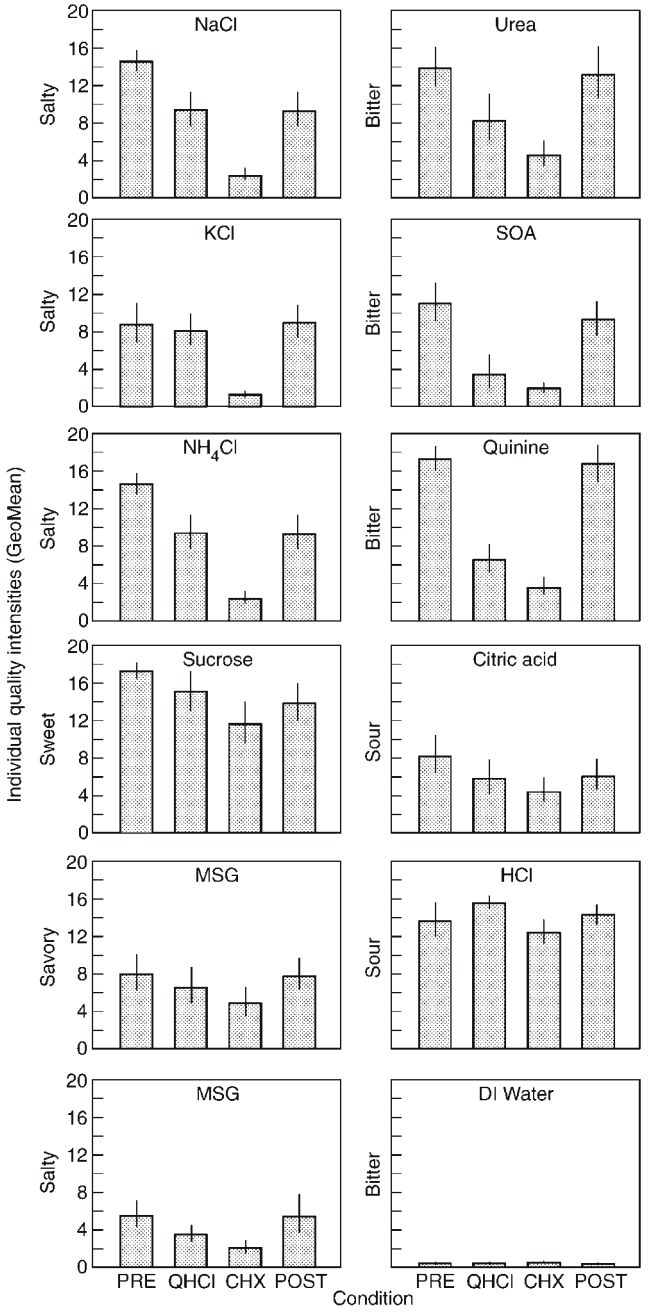

Figure 3 The axes and testing conditions are the same as in Figure 2, except that geometric means of individual qualities ± geometric SEs are depicted instead of total intensities. Although the saltiness, sourness, savoriness, sweetness and bitterness of each compound were measured, only the dominant qualities are displayed. For the three salts only saltiness was shown, for the three bitter tasting compounds only bitterness, for the two acids only sourness, for sucrose only sweetness, and for monosodium glutamate both saltiness and savoriness were plotted. In addition, the bitterness of filtered, deionized water was shown.

pre-rinse treatment to reduce the saltiness of NH_4Cl, although this was not significant. There were no apparent trends with the sourness or bitterness of NH_4Cl.

When the salts were analysed together with a three-way ANOVA, QHCl pre-rinses were not found to suppress the saltiness of any of the salts, while CHX pre-rinses suppressed the saltiness of each of the three salts relative to the water pre-rinse ($P < 0.001$) and relative to the QHCl pre-rinse ($P < 0.05$). Post-hoc tests for effects of pre-rinse treatment pooled across all three salts did not reveal any trend for QHCl to reduce saltiness ($P = 0.65$).

Bitter compounds. There was a main effect of treatment on the bitterness of urea [$F(3,45) = 5.85$, $P < 0.002$], SOA

[$F(3,45) = 10.92$, $P < 0.0001$] and QHCl [$F(3,45) = 14.92$, $P < 0.00001$] (Figure 3) and no effects of repetition. Pairwise comparisons revealed that CHX pre-rinses significantly reduced the bitterness of urea ($P < 0.002$), SOA ($P < 0.001$) and QHCl ($P < 0.00001$), while QHCl pre-rinses did not, although there was a non-significant trend for QHCl pre-rinses to decrease the bitterness of urea ($P = 0.25$), SOA ($P = 0.01$, n.s.) and QHCl ($P = 0.01$, n.s.).

When all the bitter compounds were analysed together with a three-way ANOVA, QHCl pre-rinses were found to suppress the bitterness of SOA ($P < 0.05$), but not urea or QHCl. The bitterness of the QHCl and urea stimuli after QHCl pre-rinses were not different from the bitterness ratings after CHX pre-rinses, which suggests intermediate bitterness reduction by QHCl. Post-hoc tests for effects of pre-rinse treatment pooled across all three bitter compounds revealed that QHCl tended to reduce the bitterness of all three bitter compounds ($P < 0.05$), as did CHX ($P < 0.001$), and the impacts of the two pre-rinse conditions on bitterness were overall not significantly different from each other ($P = 0.24$).

There were also no significant effects of either pre-rinse on the qualities of sucrose, MSG, citric acid, HCl, or deionized, filtered water, nor were there any effects on any of the qualities not highlighted above.

Discussion

Experiment 2 demonstrated that CHX pre-rinsing reduces the intensity of NaCl (by ~50%) and specifically reduces its saltiness (by ~80%), while QHCl pre-rinsing had no significant impact on NaCl saltiness. Experiment 2 further demonstrated that CHX specifically decreases the saltiness of KCl and NH_4Cl while QHCl did not. CHX appears to reduce saltiness via its pharmacological effects and not through its own bitterness, similar to amiloride's capacity to suppress sodium taste in selected rodents, since QHCl rinses had no significant impact on saltiness of any salt.

The observation that the saltiness from NaCl, KCl and NH_4Cl is suppressed by CHX in humans, implies that saltiness from various salt stimuli has a common pathway that CHX inhibits or suppresses. A common pathway for saltiness is also supported by the finding that adaptation to NaCl will cross-adapt the saltiness of several other salts, including those that do not contain sodium (Smith and McBurney, 1969). Thus, the present data provide further evidence that there is one saltiness pathway in the peripheral human taste system.

The bitter compounds, especially SOA and QHCl, were suppressed by CHX pre-treatment, while QHCl pre-treatment showed a marginally significant tendency to decrease the total intensity of the bitter compounds and more clearly suppressed their bitterness. Although CHX pre-rinses tended to suppress bitterness more than did QHCl pre-rinses, there appears to be a strong component of cross-adaptation from the strong bitter taste in addition to the CHX-specific pharmacological effects. The observation that all three bitter compounds were reduced in bitterness by CHX suggests that the pharmacological and cross-adaptation impacts of CHX are general effects on bitterness, yet these effects may be stronger for some compounds, e.g. SOA and QHCl, than for others, e.g. urea.

General discussion

When the oral cavity is rinsed with 0.12% CHX, the subsequent perception of saltiness from NaCl, KCl and NH_4Cl and bitterness from SOA, quinine and, to a lesser degree, urea, are specifically diminished in humans. The control bitter pre-rinse, QHCl, did not suppress saltiness significantly and reduced (cross-adapted) bitterness more weakly than did the CHX. The inhibition of salty taste by CHX would likely require a different biochemical action than its inhibition of bitter taste, although a single biochemical mechanism may be possible (see text below).

CHX is a symmetrical bis-bi-guanidinium-containing

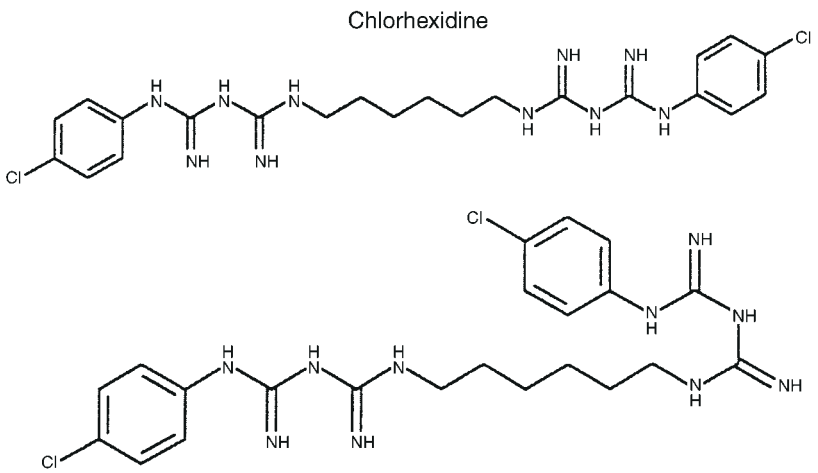

Figure 4 Two of the two-dimensional confirmers of the bis-bi-guanide molecule, chlorhexidine. Note that we are using the digluconate salt in the present studies, whereas the molecules depicted here only show the chlorhexidine cation.

compound (see Figure 4). The guanidinium group (three nitrogens bound to a single carbon sharing resonance-positive charge) has been present in many sodium channel blockers including blockers of epithelial (amiloride HCl, other related compounds) and voltage-sensitive [tetrodotoxin (TTX), saxitoxin (STX), μ-connotoxin (CTX—sea snail venom)] sodium channels. Each of these compounds contains guanidinium groups, which are understood to be their active channel-blocking constituent. Interestingly, the amino acid arginine, also a guanidinium-containing compound (and the channel-blocking component of μ-connotoxin), has been shown to enhance salty taste in humans (Riha *et al.*, 1997). Although the mechanism of amiloride blockade of epithelial sodium channels is not yet known with certainty, it is believed to bind to the cation (Na^+) selectivity filter in the external lumen of the pore (Palmer and Andersen, 1989; Schild *et al.*, 1997) and may decrease the time the channel spends in the open state (Branco and Veranda, 1992). The guanidinium-containing marine toxins TTX, STX and μ-CTX have been shown to block many types of voltage-sensitive sodium ion channels (Lipkind and Fozzard, 1994; Dudley *et al.*, 1995; Favre *et al.*, 1995). These toxins seem to block sodium flow through channels by binding their positively charged guanidinium groups to the negatively charged carboxylic acid groups on the inside of the external lumen of the sodium channels. The large size of the toxins sterically prevents sodium ions from entering the channel. This general inhibitory mechanism of guanidinium groups could well be the manner by which CHX inhibits salty taste in humans.

One major difference between these sodium channel blockers and CHX, however, is their effective modes of application. The salty taste inhibiting effect of CHX was found with a CHX pre-rinse rather than a simultaneous admixture of CHX because pilot studies did not reveal an effect of simultaneous presentation of the taste stimuli and CHX. Thus, CHX appears to need considerable 'incubation' time (tens of seconds to minutes) to block salty and bitter taste, in a manner similar to that required by *Gymnema sylvestre* to block sweet taste (Meiselman and Halpern, 1970). The precise timing of CHX delivery to block salty and bitter tastes was not the focus of the present paper, so the minimal pre-exposure time with CHX necessary for salty and bitter taste inhibition is not known. Rather, the present paper followed a conservative protocol that maximized the likelihood of finding taste-inhibiting effects. Why CHX requires pre-exposure to block salty and bitter taste is unclear. We do know, however, that the effect is not simply one of cross-adaptation, since QHCl pre-exposure did not block salty taste significantly and reduced bitterness only marginally.

As described in the Introduction, the ultimate goal of gustatory psychophysico-pharmacological studies, such as the present one, is to identify a blocker of a specific taste quality or qualities in humans that would suggest a type of transduction mechanism for them, as well as implicate the number of potential mechanisms within the quality of taste. CHX has met the first prerequisite towards this goal. That is, CHX specifically reduces salty tastes and bitter tastes and is not a general taste blocker, as it does not reduce sweet, sour, or savory tastes (as tested with the compounds in this study). The second prerequisite, however, that the pharmacological agent have a well-understood biochemical action that could potentially be effective in the mouth, has not been met. Therefore, our ability to infer a mechanism or mechanisms for salty and bitter tastes in humans is impeded by our lack of understanding of how CHX is acting on taste receptor cells in the mouth.

CHX is a disinfectant of the polyhexamethylene biguanide family (Maris, 1995). It is known to kill both Gram-positive and Gram-negative non-sporulating bacteria in the oral cavity, and so is a highly effective anti-gingival treatment (Russell, 1986; Mandel, 1994). Although the precise mechanism of how it kills bacteria is unknown, it is believed to insert itself into bacterial membranes by binding to negatively charged acid phospholipids on the membrane surface (Rolla *et al.*, 1970; Rolla and Melsen, 1975; Maris, 1995; Steinberg *et al.*, 1999), thereby reducing membrane fluidity at both hydrophilic and hydrophobic regions (Russell, 1986; Tsuchiya, 1999) and allowing for the transmembrane leak of ions, ATP and other metabolites (Iwami *et al.*, 1995). While the membrane is still intact, the membrane potential of the cell collapses and protons flow freely across the membrane (Kuyyakanond and Quesnel, 1992; Sheppard *et al.*, 1997). At this point, the cell is doomed. CHX has then effectively denatured the bacterial cell wall and ruptured the membrane (Steinberg *et al.*, 1999). Once this has occurred the membrane degrades to the degree that either: (i) at low CHX concentrations the cytoplasm and organelles leak out of the cell and it dies (Rolla *et al.*, 1970) or (ii) at high concentrations (e.g. 0.12%) the cytoplasm is coagulated and the cell dies (Loe *et al.*, 1976; Maris, 1995). Sporulating bacteria are more resistant to CHX, presumably because the surface characteristics of the spores are highly hydrophobic (Doyle *et al.*, 1984; Shaker *et al.*, 1988) and so may be resistant to the absorption of CHX via its positive charge. In order for these bactericidal mechanisms of CHX to exert their effects specifically on salty and bitter taste cells, there would have to be a constitutive difference between the membranes of salty- and bitter-sensitive cells and those of sweet-, sour- and savory-sensitive cells. Although this is possible, it seems unlikely given our understanding of human taste cell physiology.

It has also been suggested that CHX may kill bacteria via a cation-chelating mechanism that inhibits proteolytic activity of host enzymes, especially metalloproteinases (Gendron *et al.*, 1999). CHX has also been observed specifically to inhibit membrane-bound ATPase in selected bacteria (Harold *et al.*, 1969; Kuyyakanond and Quesnel, 1992). These observations suggest that CHX might have a negative impact on membrane-bound enzymes necessary

for transduction, particularly in bitter tastes, since bitterness transduction depends on enzymes acting on membrane-bound proteins, in contrast with salty taste which is believed to occur when ions pass directly though ion channels in salty-taste-sensitive cells. Why sweet-sensitive cells would be spared by this enzymatic inhibition, however, is less clear. Perhaps the activity(ies) of phospholipase C, protein kinase C, or phosphodiesterase, all bitter transduction enzymes, is inhibited by CHX while adenylate cyclase, which has been implicated in sweet taste but not in bitter taste, is not (Lindemann, 1996a,b).

The observation that CHX does not block all taste qualities provides further evidence for the independence of the taste qualities. Prior examples include compounds such as lactisole and *Gymnema sylvestre* that block sweetness without affecting other qualities (Meiselman and Halpern, 1970; Lindley, 1991; Johnson *et al.*, 1994; Sclafani and Perez, 1997; Schiffman *et al.*, 1999) and large-anion sodium salts that reduce bitterness without affecting other qualities (Breslin and Beauchamp, 1995). There are presently no known specific blocking agents for sourness or savory/umaminess in humans. The blockade of both saltiness and bitterness by CHX suggests that the two qualities are not totally independent. This is not the first time this association has been reported. Von Skramlik (Von Skramlik, 1963) observed that certain topical anesthetics, such as Eucupin, Diocain and Psicain-*new*, when applied to the tongue would greatly reduce bitterness and saltiness perception and only minimally reduce sweetness and sourness. Saltiness and bitterness could be linked physiologically in such a way that channel blockers (possibly CHX) would specifically inhibit saltiness and bitterness via action on a single mechanism. For example, a non-specific cation channel on salty-taste-sensitive cells may be the direct pathway for transducing salty taste, while the same (or similar) cation channels are involved with down-stream transduction cascades of bitterness in bitter-taste-sensitive cells.

Future directions

This paper presents the first evidence that salty taste can be reduced in humans via an acute, oral, pharmacological pre-rinse. Although there is a strong parallel between the action of oral amiloride in certain rodents on sodium-specific taste and the action of oral CHX on perceived saltiness in humans, there is an important difference between the two in that amiloride's action on epithelial cation channels is known and the action of CHX on epithelial cation channels is not. In addition, CHX is one of the few compounds that has been shown to reduce bitterness perception in humans. Many questions remain unanswered about the salty- and bitter-taste-reducing properties of CHX.

Saltiness inhibition

We have provided evidence that the saltiness of NaCl, KCl and NH_4Cl was greatly reduced by CHX (see Figure 3). Other salty-tasting compounds need to be tested to determine whether CHX would inhibit any source of saltiness or whether its blocking effects are specific to these three salts. One implication of the present saltiness reductions by CHX is that the saltiness of NaCl, KCl and NH_4Cl is inhibited because salty-taste transduction in humans occurs via an epithelial CHX-sensitive cation channel with a selectivity filter that permits Na^+, K^+ and NH_4^+ ions to pass through it. This hypothesis will require biophysical and electrophysiological tests with CHX on human gustatory tissue for validation. Another outstanding question concerns why CHX did not block all of the salty-taste intensity of the 300 mM NaCl solutions. Perhaps, as has been argued for amiloride's partial NaCl blocking effects, some of the cations pass paracellularly through tight junctions to access basolateral cation channels on salty taste cells where CHX cannot follow to block the channels (Delwiche *et al.*, 1999). A full concentration–response function for NaCl and CHX is needed to confirm that CHX will not block all of the saltiness when applied at higher concentrations or when mixed with lower concentrations of NaCl.

Bitterness inhibition

CHX clearly reduces the bitterness of urea, SOA and QHCl beyond the reductions of intensity-matched quinine rinses. Since there is evidence that urea and QHCl have different bitter-taste transduction mechanisms (McBurney *et al.*, 1972) the question arises whether CHX would reduce the bitterness of all bitter-tasting compounds. What would such a finding suggest about bitterness coding, given that there is considerable evidence that there are multiple bitter transduction mechanisms (Lindemann, 1996a,b)? Could CHX be acting on downstream bitterness transduction events rather than on primary membrane-bound 'classical' receptors? Or, since SOA was suppressed to a greater extent than was urea, perhaps CHX is acting on specific receptor mechanisms. A second line of questioning will need to pursue the different roles of cross-adaptation and pharmacological inhibition of bitterness by CHX. Although quinine was as bitter as CHX, it did not reduce the bitterness of other compounds to the same degree; yet, CHX molecules are known to linger in the oral tissue and saliva [which is why, in part, it is a highly effective anti-bacterial treatment (Russell, 1986)]. As a result, could CHX be more effective than QHCl because it has greater access-time to cells to cross-adapt them? Could a cross-adaptation effect also explain why bitterness was not completely inhibited by CHX, since cross-adaptation is rarely complete? The answers to these questions will help determine whether chlorhexidine is a useful pharmacological tool for understanding the biochemical mechanisms that underlie human salty and bitter taste.

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